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Comparison of Different Methods for Assessing Chlorophyll Content in Citrus

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Abstract

The content of chlorophyll-Chl present in green vegetables is strongly associated with the momentary state of the plant photosynthesis. Conventionally, determination of chlorophyll is by extraction with organic solvents and determining a spectrophotometer, and this method expensive, laborious and moreover a destructive method. Thus, the use of portable equipment has been used instead, as they allow studies without destruction of leaf tissue and obtaining instantaneous measurements. Thus, the aim of correlating the chlorophyll results obtained in the laboratory with Falker Chlorophyll Index (FCI) obtained by the ClorofiLog in different citrus rootstocks, as well asits relationship to soil moisture content variation. Positive simple correlations (linear Pearson correlation) were obtained for chl a, b and total, with the lowest correlation values observed for chl β, which ranged from 0.470-788 among the analyzed rootstocks. The coefficients of determination for the three variables chl α, β and total $\alpha+\beta$ showed a better fit by the polynomial regression model in all analyzed rootstocks; in general, the best results were for chl α, ranging from 0.7093-0.8551. The results indicate the usefulness of the ChloroflLog apparatus in the indirect determination of chlorophyll content in citrus.

Keywords: indirect determination of chlorophyll content, Falker Chlorophyll Index, Volkamer lime, Santa Cruz Rangpur lime, soil moisture, Cleopatra mandarin, Tropical Sunki mandarin, TSK × TRENG 256

1. Introduction

The Citrus is a very important crop in the Brazilian economy, being cultivated throughout the country, especially in the states of São Paulo, Bahia and Sergipe (IBGE, 2016). The citrus production in Northeast Brazil focuses on the tableland, where rainfall distribution is very uneven, with records of long periods of drought, a factor that affects agricultural productivity and causes extensive damage to producers (E. Coelho, Coelho Filho, Simões, & Y. Coelho, 2006).

One of the main processes related plant productivity is photosynthesis, and among the factors related to the photosynthetic efficiency and hence growth and adaptability to different environments is chlorophyll, present in all green vegetables (Engel & Poggiani, 1991). These chloroplastid pigments are important in this context because they are responsible for light used in photosynthesis and are essential in the conversion of light radiation into chemical energy ATP and NADPH (Batista, Araújo, Antunes, Cavatte, Morães, Martins, & Damatta, 2012). The Chlorophylls ($\alpha + \beta$) they are not only pigments responsible for the color expression in plants but are also indicative of photosynthetic efficiency of plants and therefore its primary productivity, since they control the amount of solar radiation absorbed by plants (Engel & Poggiani, 1991; Blackburn, 2007).

In this case, the stress by drought can lead to loss of chlorophyll and decline in photosynthetic capacity (Silva, Santos, Vitorino & Rhein, 2014). Thus, the amount and composition of pigments present in plants can be used as parameters to evaluate the stress level in plants and their respective tolerances (Codognotto et al., 2002; Jabeen, Shahbaz & Ashraf, 2008).

Quantitation of chlorophyll content is normally carried out by extraction using different organic solvents such as acetone, ether, dimethylsulfoxide or methanol (Cruz et al., 2007), followed by the standard determination based on the absorbance of light by the pigments (Arnon, 1949). However, besides the variation in results depending on the type of solvent used (Ritchie, 2008), this method is very laborious.

Thus, portable meters have been developed that determine chlorophyll content, based on optical characteristics of the sheets (Argenta, Da Silva, & Bortoline, 2001). This indirect method calculates the chlorophyll content from the amount of light transmitted through a sheet at two or three wavelengths (depending on device brand) at different absorbencies, generating a chlorophyll content that corresponds to the amount of chlorophyll α and β (Argenta et al., 2001).

One such device is the ClorofiLog, CFL 1030 model produced by Falker Agricultural Automation. The clorofiLog measures the absorption of light by the sheet at specific wavelengths: 635, 660 and 880 nm and absorption from the relations in the different frequencies is determined Falker Chlorophyll Index (FCI) (Falker, 2009). This equipment is of recent use in relation to SPAD, chlorophyll meter of Japanese origin (Barbieri junior, Rossiello, Silva, Ribeiro, & Morenz, 2012.), which was already employed in various jobs, including citrus (Jifon, Syvertsens, & Whaley, 2005; Vale & Prado, 2009). The use of the devices in determining the chlorophyll content's main advantage the fact that they are more practical and fast (Mielke, Schaffer, & Li, 2010), especially by producers in the field (Leonardo, Pereira, Silva, & Costa, 2013).

Thus, whereas the chlorophyll content may vary in response to environmental changes and their analysis from the non-destructive method may represent an important tool in agriculture. We sought to compare the chlorophyll content obtained by the direct method by dimethyl sulfoxide and Chlorophyll content in different Falker rootstock citrus and its relationship to moisture content variation in soil.

2. Materials and Methods

The work was carried out on the farm Coconut Lagoon, Rio Real, BA, (geographical coordinates: 11°34′25″S and 37°52′58″W, altitude 182 m), north coast of Bahia, located at Coastal Board, with flat topography wavy soft and predominance of Oxisols Yellow and Yellow Argissolos presenting the cohesive character in natural conditions. The property consists of some experimental blocks linked to the Federal University of Bahia Reconcavo. The orchard has analyzed eight years of implementation and during the search the cultural treatments were limited in weed control mechanically.

The experiment consisted in the evaluation of five combinations Cup rootstock citrus, and orange cup “Pera” grafted on Santa Cruz Rangpur lime, Volkamer mandarin, Tropical Sunki mandarin, Cleopatra mandarin and Indian lime (TSK × TRENG 256) monthly analyzes were performed for ten months (March to December, 2017).

The Falker Chlorophyll Index (FCI) was determined by reading with chlorophyll meter, ClorofiLog CFL 1030 model, in the median part of the leaf, on the adaxial surface, on three leaves per plant. Then he proceeded to the extraction 5.0mm diameter disks with the aid of a manual punch on three sheets (1disco/sheet). The collected material was transferred to glass tubes previously coated with aluminum foil containing 5 ml of dimethylsulfoxide (DMSO). The tubes were appropriately sealed with their lids. The protocol used for the extraction and quantification was to dimethylsulfoxide (DMSO), as described in Barnes, Balaguer, Manrique, Elvira, and Davison (1992).

In the laboratory, the vials were kept in the dark for 48 hours at room temperature, after which the extracts were analyzed in a spectrophotometer, absorbance at 649 and 665nm. The estimated concentrations of chlorophyll α and β was made from the following equations: Chl α (μg mL^{-1}) = $12.7A_{663} - 2.69A_{645}$; Chl β (μg mL^{-1}) = $22.9A_{645} - 4.68A_{663}$; total chl = (Chl α + Chl β).

To monitor the soil water potential installed in each plant analyzing a TDR probe different depths in five 0-0.7; 0.7 to 0.17-0.17 to 0.47; from 0.47 to 0.87; from 0.87 to 1.07 cm, according to the depths of horizons. The readings recorded in the TDR soil moisture based on calibration equation proposed by Ledieu et al. (1986) (1) inserted into the machine:

$$\theta = 0.1138\sqrt{Ka} - 0.1758 \tag{1}$$

Where, θ = volumetric water content (cm^3 cm^3); Ka = apparent dielectric constant of the soil.

The apparent dielectric constant of soil was obtained for each humidity value recorded in the TDR using Equation 2:

$$Ka = \left(\frac{\theta + 0.1758}{0.1138}\right)^2 \tag{2}$$

Calibration of the TDR probes used to record the soil moisture in the field was made using soil samples from each horizon with undeformed structure, which were saturated in the laboratory after inserting TDR probes therein. After that θ were simultaneously made to read the TDR for about 30 days natural drying of samples, allowing to obtain Ka based on equations 1 and 2, and their determined gravimetrically θ (θ = Ug × Ds). The data θ determined at each horizon of the soil profile and recorded Ka (Equation 2) were adjusted to a cubic polynomial model (Equation 3), for processing of the field data θ (Table 3):

$$\theta = aKa^3 - bKa^2 + cKa^3 - d \quad (3)$$

The experimental design was a split-plot, with three replicates and five treatments, with each replicate comprising a plant. The following treatments were examined: crownorange "Pera" grafted on Cravo Santa Rangpur lime, Volkamer mandarin, Tropical Sunki mandarin, Cleopatra mandarin, and Indian lime (TSK × TRENG 256). The data obtained in evaluations were subjected to a simple correlation analysis (Pearson correlation) between the FCI values and chlorophyll content. Additionally it was carried out linear regression polynomial and with 5% significance using the software Sisvar v. 5.3 (Ferreira, 2008). After it was also realized simple correlation (Pearson's linear correlation) between soil moisture content and the determined levels of chlorophyll in the laboratory as well as in relation to Falker Chlorophyll index.

3. Results and Discussions

The results of simple correlation (linear correlation Pearson) to the determined levels of chlorophyll in the laboratory and the results of the Falker Chlorophyll Index (FCI) (Table 1), show a high correlation between the values for chl α variable, and the smallest correlation value obtained was for the Volkamer lime rootstock and the highest value for the indian lime (TSK × TRENG 250), with respective values (0.809 and 0.897). These results indicate the possibility of using the handset to replace laboratory analysis without major damage, especially for most rootstocks and especially to follow this culture in agriculture.

For the results of chl *β*, there is a lower correlation, and rootstocks with the highest correlations were respectively Cleopatara mandarin (0.788) and Tropical Sunki mandarin (0.699). The values found for total chlorophyll α+β, varied between (0.766) for Indian lime (TSK TRENG 250) and (0.914) for the Cleopatara mandarin.

Table 1. Pearson correlation coefficients (Pearson linear correlation) between the values of chlorophyll α, β and the total (α+β) extracted by DMSO in relation to Falker index for different rootstock (Penx) citrus

Penx	Correlations		
	Chl α	**Chl β**	**Total Chl (α+β)**
L. Volk	0.809	0.543	0.806
L. CSC	0.825	0.561	0.831
L. Indian	0.897	0.470	0.796
T. Sunki	0.873	0.699	0.869
T. Cleo	0.862	0.788	0.914

Note. Penx: rootstock; Chl α: chlorophyll α; Chl β: chlorophyll β; Total Chl: total chlorophyll (α+β); L. Volk: Volkamer lime; L. CSC: Santa Cruz Rangpur lime; L. Indian: Indian lime (TSK × TRENG 256); L. Sunki: Tropical Sunki mandarin; L. Cleo: Cleopatra mandarin.

Since the coefficient of determination (R^2) found for each rootstock from the linear regression model (Figure 1) and polynomial regression model (Figure 2), there is a relative increase in the linear model for polynomial model, indicating that the latter had a better correlation adjusting for all variables, but especially for the chl α and total chl. Jifon and Whaley (2005) also observed reductions in the correlation coefficient obtained by linear regression in citrus, compared to the polynomial regression model, attributing this resultlow efficiency of light absorption by leaves with high content of Chl. Although slightly smaller, coefficients for determining the chl α through polynomial regression model were closer to the linear Pearson correlation values than values obtained by the linear regression model.

The coefficients of determination (R^2) by linear regression model for chl α from 0.65 in the Volkamer lime rootstock, 0.80 in Indian lime (TSK × TRENG 256); linear coefficient of determination for the total chlorophyll α+β change between 0.63 and 0.83 respectively for Indian lime (TSK × TRENG 256) and Cleopatra mandarin rootstocks. When evaluating a variety of limes, Jesus and Marenco (2008) found a linear coefficient of determination of 0.80, by SPAD index, for both chl α and total chlorophyll α+β; the majority of the rootstocks of the present study presented approximate determination values.

The coefficients of linear determination to chlorophyll β found in this study were very low, ranging from 0.22 to the Indian lime (TSK × TRENG 256) and 0.62 for cleopatra mandarin; most rootstocks differ widely from the value found by Jesus and Marenco (2008) corresponding to 0.76 in variety of lemon. However, these values are similar to that found by Neves et al. (2005) on cotton, which found a lower correlation 0.48 to chl β and 0.79 for the chl α, attributed this difference to the fact that the second peak of absorption of red light by chlorophyll α (663 nm) plus be greater than that of chlorophyll β, it is very close to the wavelength emitted by SPAD-502 (650). This situation may apply to the results in the present study, despite being Falker Chlorophyll Index (FCI) and not the SPAD index. However, ClorofiLog also emits peak absorption (660 nm) closer to that of chl α than chl β.

In the regression polynomial model, the coefficient of determination values (R^2) for chl α variable ranged from 0.71 and 0.85 on Cravo Santa Rangpur lime and Tropical Sunki mandarin rootstocks, respectively. These values are similar to values found by Jifon and Whaley (2005) for some varieties of citrus polynomial model by correlating the chl and SPAD index. Barbieri Junior et al. (2012) found chl content of forage grass Tifton correlations and the FCI values (0.64 and 0.79), all of which are within the range of values found in this work, even though they are in very different species. Due to the limited publication of papers with FCI, the data were compared to more SPAD index, because it is similar principles.

The linear correlation results yesple (correlation Pearson) (Table 2) between soil moisture content and chlorophyll α, β and total (α+β) determined in laboratory as well as the FCI showed a greater correlation of soil moisture with FCI than the determined levels of chlorophyll laboratory; the correlation amplitudes varied between (0.667 to 0.783) to chl α, (0.540 to 0.834) chl β (0.676 to 0.966) total chl (α+β) in the results of FCI (0.540 to 0.706) to chl α, (0.320 and 0.670) to chl β (0.585 to 0.813) to total chl (α+β) determined in the laboratory. In general, higher soil moisture at either correlations laboratory method as the FCI were observed for the total chl. Silva et al. (2014) found that the soil moisture variation changed the chl α and β in the total sugar cane, confirming the correlation between these variables.

Among the rootstocks analyzed, it is found that the Volkamer lime and Tropical Sunki mandarin were those with the highest correlations for most variables, both the laboratory method as for the FCI.

Table 2. Pearson correlation coefficients between soil moisture and chlorophyll α, β and total (α+β) extracted with DMSO and Chlorophyll index obtained by Falker (FCI) for different rootstock (Penx) citrus

Penx	Chl DMSO (μg mL^{-1})			FCI		
	Chl β	**Chl b**	**Total Chl (α+β)**	**Chl α**	**Chl β**	**Total Chl (α+β)**
L. Volk	0.674	0.605	0.813	0.705	0.834	0.966
L. CSC	0.540	0.410	0.585	0.668	0.540	0.755
L. Indian	0.651	0.320	0.589	0.667	0.560	0.676
T. Sunki	0.706	0.487	0.747	0.783	0.803	0.892
T. Cleo	0.569	0.670	0.646	0.680	0.670	0.681

Note. Penx: rootstock; Chl α: chlorophyll α; Chl β: chlorophyll β; Total Chl: total chlorophyll; L. Volk: Volkamer lime; L. CSC: Santa Cruz Rangpur lime; L. Indian: Indian lime (TSK × TRENG 256); T. Sunki: Tropical Sunki mandarin; T. Cleo: Cleopatra mandarin.

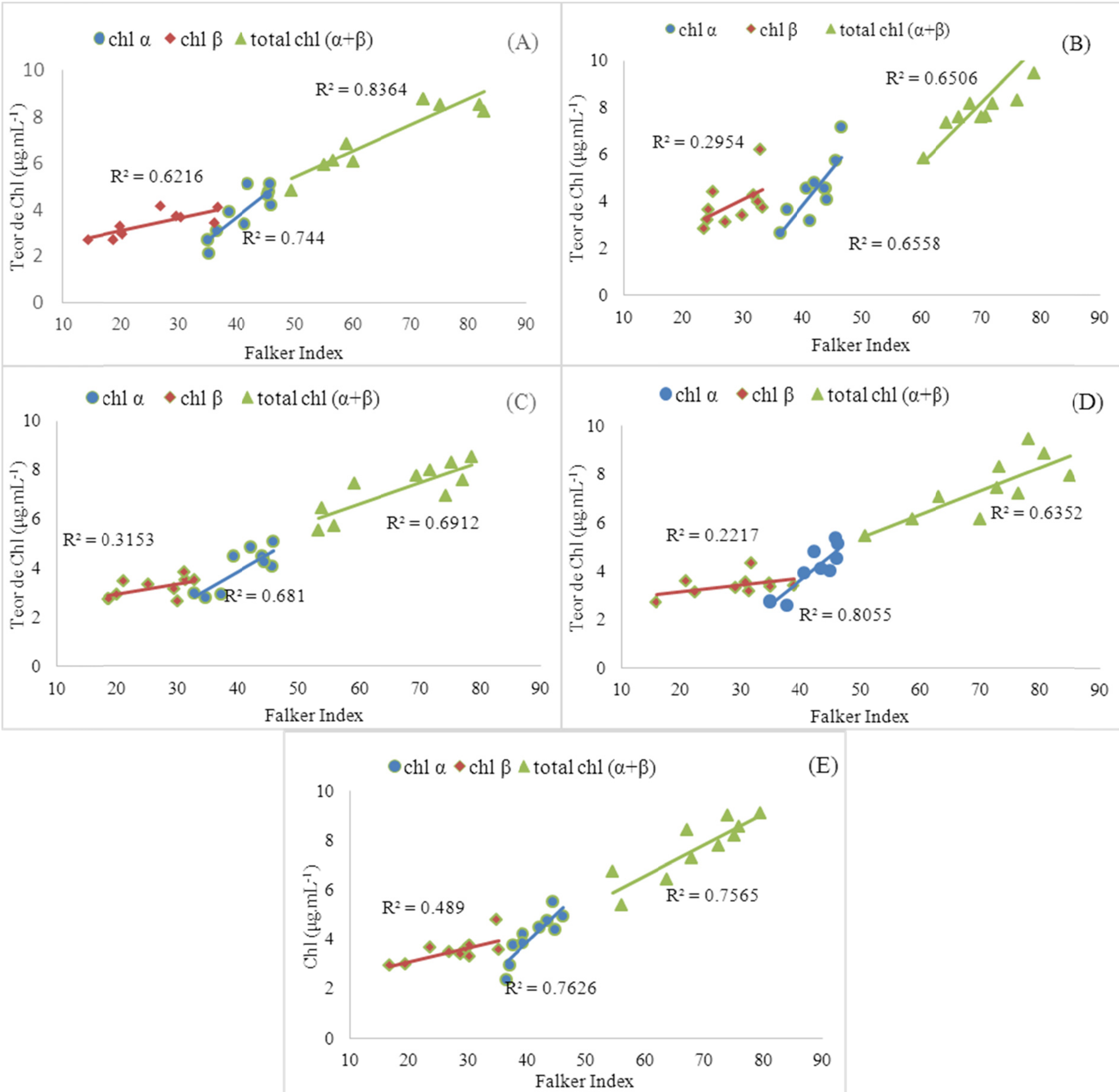

Figure 1. Linear relationship between the contents of chlorophyll α, β and total (α+β) extracted with DMSO and Falker index for Cleopatra mandarin (A); Volkamer lime (B), Santa Cruz Rangpur lime (C); Indian lime (TSK × TRENG 250) (D), and Tropical Sunki mandarin (E). chl: chlorophyll

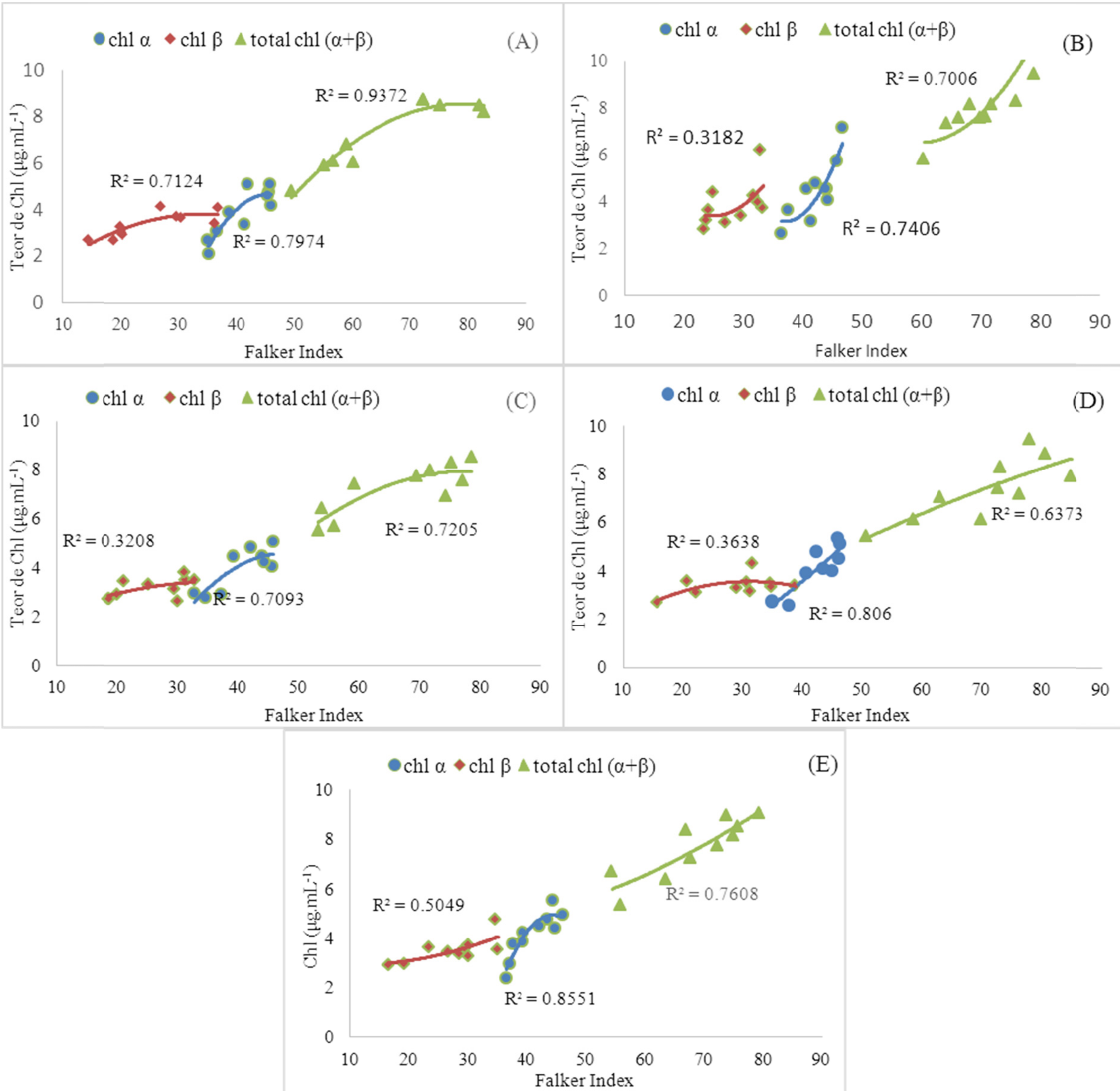

Figure 2. Polynomial relationship between the contents of chlorophyll α, β and total (α+β) extracted with DMSO and Falker index for Cleopatra mandarin (A); Volkamer lime (B), Santa Cruz Rangpur lime (C); Indian lime (TSK × TRENG 250) (D), and Tropical Sunki mandarin (E). chl: chlorophyll

4. Conclusions

(1) The results of simple polynomial regression and linear correlation indicates the possibility of using the portable device instead laboratory analyzes, especially in the management of the citrus crop.

(2) The polynomial regression model was shown to be the most suitable for analysis of correlation between the chlorophyll content in citrus and ICF, as compared to the linear regression model.

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